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Author(s)	Inai, Shinya; Fujikawa, Katsumi; Masaoka, Tooru et al.
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Deficiency of the Hydrazine Sensitive Component of Complement in a Patient with Chronic Myelogenous Leukemia*

SHINYA INAI, KATSUMI FUJIKAWA, TOORU MASAOKA AND HISAO TAKAHASHI

Center for Adult Diseases, Higashinari-ku, Osaka

KAZUYOSHI NAGAKI

*The 3rd Department of Internal Medicine,
Osaka University Medical School, Osaka*

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SUMMARY

A diminution of serum complement activity was found in a patient with chronic myelogenous leukemia. It was concluded that a very low titer of the hydrazine sensitive component (HSC) of complement was responsible for this phenomenon. The possible relationships between the increase in the white cell count and HSC activity are discussed.

INTRODUCTION

Although many observations on the complement (C') levels in patients with various diseases have been reported, there have been few measurements of the levels of the individual components of C'. In the course of studies on the bactericidal activity of sera in various diseases, it was found that the serum of a patient with chronic myelogenous leukemia had low C' activity, although three patients with leukemia (two acute myelogenous leukemias and one acute lymphatic leukemia) and other patients with neoplasma showed normal C' levels. The present paper described the changes in the activities of C' and HSC during the course of the disease and their possible relationship to clinical findings in this patient.

The term hydrazine sensitive component (HSC) refers to the component of C' (C'4, C'3b, C'3c.....) which is destroyed by treatment with hydrazine.

MATERIALS AND METHODS

1. Complement

Blood from healthy guinea pigs was obtained aseptically by cardiac puncture without anesthesia. Blood from the patient and normal human was taken aseptically by venous puncture. After standing about one hour at room temperature the sera were drawn off, centrifuged and either used on

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the same day or stored at -20°C before use.

2. *Guinea pig complement lacking HSC (R4)*

To 1.0 ml of guinea pig serum was added 0.25 ml of 0.075 M hydrazine. The mixture was incubated for one and one-half hours and then neutralized with 0.25 ml of 0.075 M HCl. Saline was added to give a final dilution of one fifth with respect to the original serum volume (Kabat and Mayer, 1961). The amounts of R4 used in these experiments were neither lytic nor anticomplementary.

3. *Sensitized sheep erythrocytes (EA)*

Sheep blood was stored with an equal volume of Alsever's solution at 4°C .

Immediately before use, blood cells were washed twice with physiological saline and once with veronal saline buffer of pH 7.4 containing 0.00015M Ca^{++} and 0.0005M Mg^{++} . They were resuspended in the same buffer. Veronal buffer containing 8 units of hemolysin per ml was added to an equal volume of suspension of washed cells containing 10^9 cells per ml, and the mixture was incubated in a water bath at 37°C for 10 minutes for sensitization.

4. *Titration of complement*

This was estimated by the addition of varying quantities of an appropriate dilution of serum to 1.0 ml of EA. Veronal buffer was added to a final volume of 3.0 ml. The tubes containing the mixtures were incubated in a water bath at 37°C for 30 minutes. At the end of this period, the tubes were placed in an ice bath, 2.0 ml of chilled saline were added and the tubes were centrifuged at 3,000 rpm for 10 minutes. The optical density of the supernatant fluids were analyzed for oxyhemoglobin at a wave length of $530 \text{ m}\mu$ in the Hitachi photoelectric photometer. Fifty per cent hemolytic units of C' activity ($\text{C}'\text{H}_{50}$) were calculated.

5. *Titration of hemolytic activity of complement with R4 (HSC)*

This was estimated by the addition of varying quantities of an appropriate dilution of serum and 0.3 ml portion of R4 to 0.4 ml portion of EA. Veronal buffer was added to a total volume of 2.0 ml. After 30 minutes incubation at 37°C in a water bath, the tubes containing the mixtures were centrifuged to remove unlysed cells. Oxyhaemoglobin in the clear supernatant fluids was analyzed photometrically at a wave length of $530 \text{ m}\mu$ in the Coleman junior spectrophotometer. Fifty per cent hemolytic units of HSC in the sera were calculated, and tentatively designated as HSC activity.

The normal ranges of $\text{C}'\text{H}_{50}$ and HSC activities obtained by these methods were 50 to 100 units per ml, and 4,000 to 12,000 units per ml, respectively.

RESULTS

A twenty year old woman was admitted to this hospital on June 9, 1962. She had been diagnosed as having chronic myelogenous leukemia about one year previously.

On admission, there were no abnormal physical signs except for hepatomegaly of 3 finger-breadths below the right costal margin and a splenomegaly of 4 finger-breadths. The urine gave negative tests for protein and sugar. Examination of the blood showed a red cell count of 4.88×10^6 , hemoglobin: 15.25 gm per 100 ml, white cell count: 34,200, with 11.5% myeloblasts, 6.5% promyelocytes, 13.5% myelocytes, 4.5% metamyelocytes, 12.5% band forms, 13.5% neutrophils, 2% eosinophils, 26.5% basophils and 8.5% lymphocytes. The platelet count was

860,000. The bleeding time was prolonged more than 30 minutes and the clotting time was 7 and a half minutes. Bone marrow aspiration revealed a total cell count of 420,000, with 7.7% myeloblasts, 5.7% promyelocytes, 18.3% myelocytes, 8.7% metamyelocytes, 9.6% band forms, 20.2% neutrophils, 0.2% eosinophils, 19.7% basophils, 1.9% lymphocytes, 5.4% normoblasts and 0.2% plasma cells, and an L/E ratio of 16.8. Liver function tests and serum electrolytes were within the normal ranges.

Plasma electrophoresis showed that the total protein was 6.0 gm per 100 ml, with 49% albumin, 10% α -globulin, 9% β -globulin, 45% γ -globulin and 7% fibrinogen. The urea nitrogen was 9.0 mg per 100 ml.

She was treated with prednisolone, busulfan and 6-mercaptopurine. However, blood transfusion was not performed since the red cell count and hemoglobin contents were within the normal range.

Table 1. Blood Picture of the Patient

Date	R.B.C. $\times 10^4/\text{cmm}$	Hb gm/dl.	C.I. $/\text{cmm}$	W.B.C. $/\text{cmm}$	Myelo- blast			Pro- myelo- cyte			Myelo- myelo.			Juvenile Segment			Eosin. Baso. Mono. Lymph.		
					1	2	3	1	2	3	4	5	6	7	8	9	10	11	12
12/27/62	402	11.75	0.93	11,100	39	0	0	0	7	28	1	0	0	6	19				
1/7/63	449	13.5	0.95	13,700	28	2	3	2	3	35	0	1	1	7	20				
1/11/63				22,100	23	0	5	4	5	25	0	0	0	3	36				
1/21/63	501	13.95	0.89	40,300	46	1	8	5	4	18	0	0	0	3	15				
1/28/63	445	13.75	0.98	75,500	13.5	5.5	17.5	13	14	17.5	0	0.5	7.0	11.5					
2/6/63	429	11.75	0.87	154,000	5.5	1.5	14.5	26.5	9.5	32.5	0	0	4.0	5.0					
2/8/63				113,500															
2/18/63	347	9.0	0.81	18,300															
3/1/63	340			4,800	21	3	9	1	3	25	0	1	1	7	29				
3/9/63				6,900															
3/22/63				50,800	14	5	16	6	19	13	0	0	0	18	9				
3/28/63				7,300	10	0	2	0	8	57	0	0	0	3	20				
4/2/63	290	8.25	0.89	1,900	23	0	4	3	3	25	0	3	3	3	36				
5/13/63	313	10.0	1.02	59,500	2	3	10	20	20	33	0	0	3	3	14				

The first and the second titration of C' were done on Jan. 10 and 22. In each experiment, hemolysis of sensitized sheep cells was not seen even with 0.5 ml of undiluted serum, indicating that C' activity was almost nil. At the times of titration, the white cell counts were 22,100 and 40,300 respectively. The differential count of white cells is shown in Table 1. The disappearance of C' activity was further investigated by the following procedures. First, it was confirmed that this serum was not anticomplementary to human and guinea pig C'. Second, the activity of each component of C' was examined with the respective reagents. The activities of C'1, C'2, and C'3 measured with reagents were found to be fully maintained. However, as indicated in Table 2, a remarkable decrease of HSC

activity, that is to 8 units per ml, was observed. In contrast, the HSC activity in normal persons was found to be in the range of 4,000 to 12,000 units per ml. Since 8 units of HSC is practically negligible, the disappearance of C' activity is presumably due to the marked reduction in HSC activity. Titrations of C' and HSC activities were carried out several times during the clinical course of the patient. As indicated in Tables 1 and 2, C'H₅₀ was almost nil on Jan. 28. It

Table 2. Complement and Hydrazine Sensitive Component Levels of the Patient

Date in 1963	C'H ₅₀	HSC
1/10	2>	—
1/22	2>	8
1/28	2>	—
2/ 1	3.2	15
2/ 7	44.5	235
2/18	39.0	323
3/ 1	34.5	32
3/ 9	4.5	10
3/29	49	910
5/13	55.6	3,750

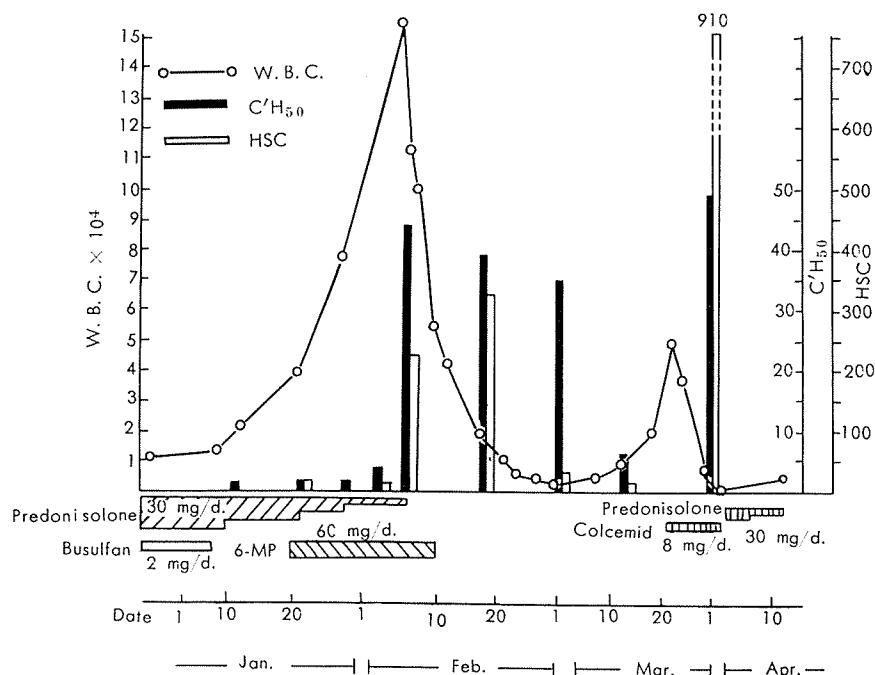


Fig. 1. Clinical Course, Showing the White Cell Counts, Complement and HSC Levels.

increased to 3.2 units on Feb. 1, when the numbers of white cells had rapidly increased to 98,500. The white cell count reached a maximum of 154,000, on Feb. 6. $C'H_{50}$ had been restored to 45 units on Feb. 7, but the HSC activity was still below the normal range. Then the white cell count gradually decreased but had returned to normal by Feb. 25. In the following two weeks no significant change in the white cell count was observed. On March 9, $C'H_{50}$ and HSC activities had again markedly decreased, and about two weeks later the white cell count had increased to 50,800. On May 13, $C'H_{50}$ and HSC activities were at almost the normal levels despite a considerable increase in the white cell count.

As is shown in Fig. 1, the fact that the activities of C' and HSC were relatively independent of the drugs used during the treatment, indicates that these drugs may have only minor effects on the reduction of C' and HSC activities.

DISCUSSION

A number of investigations have been carried out on C' activity in various diseases and these are summarized in the recent review by Osler (1961). It has been accepted generally that a diminution in C' level was observed in renal diseases, especially in the active stage of acute nephritis.

Recently, Nastuk *et al.* (1960) observed a decrease in C' activity in patients with myasthenia gravis. Baltch *et al.* (1960) reported a similar phenomenon in chronic lymphatic leukemia complicated by pneumonia. However, the authors suggested that the reduction in C' activity might be due to pneumonia. Beeson and Rowley (1959) suggested that the peculiar susceptibility of kidney to infection was probably due to inactivation of C'4 by ammonia accumulated in the kidney, but they did not demonstrate a decrease in C' nor in its components in infectious diseases of the kidney.

The present patient with chronic myelogenous leukemia showed an interesting fluctuation of C' and HSC activities during the course of the disease. As described above, a marked reduction in C' activity of the serum was observed five times in this patient. Especially in the first three titrations C' activity was almost nil. The serum specimens used in these titrations were not anticomplementary to human and guinea pig C'. Titration of the components of C' with reagents disclosed a remarkable loss of HSC activity which is attributable to a deficiency in the components of C' sensitive to hydrazine. It is known that the component of C' which is sensitive to hydrazine or ammonium hydroxide is C'4. Recently, studies on the components of C' showed that the titration of the component with R reagents is dubious from experimental and theoretical stand points. Because, C'3 from guinea pig serum was separated into four (Nishioka and Linscott, Linscott and Nishioka, 1963) or five (Nelson and Inoue, 1963) subfractions, and two of these subfractions (C'3b and C'3c) were reported to be destroyed by

treatment with hydrazine or ammonia (Linscott and Nishioka, 1963). However, the details of the methods of titration of these subcomponents have not been reported and a satisfactory technique for measuring C'4 activity without using R4 has not been reported yet. Moreover, deficiency of the subcomponent of C'3 in serum cannot be detected by R3, because some of these subcomponents are retained in R3 which was prepared by zymosan, as indicated in the previous paper (Inai *et al.*, 1961). Therefore, when using R4 reagents, the overall activities of C'4, C'3b and C'3c of serum are measured and it is uncertain whether C'4 or any of the subcomponents of C'3 were involved in the decrease in C' level of this patient.

The deficiency of C' activity in this patient was not considered to be hereditary, since, as far as tested, C' activity in her family was almost normal.

Diminution of C' activity may develop under various clinical conditions, such as nutritional disturbances and complications following infectious diseases, renal diseases or allergies. However, during the clinical course of the patient there were no clinical signs consistent with these conditions. Obviously, it is most important to rule out the effect of medical treatment upon the activities of C' and its components. However, as described above, busulfan, prednisolone, colcemid and 6-mercaptopurine apparently do not play a significant role in the diminution of C' and HSC activities. It is conceivable that the changes in the white cell count might have a significant relation to the diminution of HSC activity, because when its activity decreased markedly there was a subsequent gradual increase in the white cell count, and when the increase in the white cell count ceased the HSC activity increased. Further investigations on the relationship between the changes in the white cell count and HSC activity is now being undertaken.

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